16:43:00 OCA PAD INITIATION - PROJECT HEADER INFORMATION 07/12/89

Active

Project #: G-33-W08 Cost share #: Rev #: 0
Center # : 10/24-6-R5983-8A0 Center shr #: OCA file #:
Work type : RES
Contract#: 2 R01 HL28167-08 Mod #: Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N
Main project #:

Project unit: CHEM Unit code: 02.010.136
Project director(s):
MAY S W CHEM (404)894-4052

Sponsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
Sponsor/division codes: 108 / 001

Award period: 890701 to 900630 (performance) 900930 (reports)

Sponsor amount	New this change	Total to date
Contract value	248,551.00	248,551.00
Funded	248,551.00	248,551.00
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: NOVEL ANTIHYPERTENSIVES: RATIONAL DESIGN AND EVALUATION

PROJECT ADMINISTRATION DATA

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BETHESDA, MD 20893

Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT

Administrative comments -
PROCESSED INITIATION. YEAR 8 OF 12 YEAR AWARD. CONTINUATION OF PROJECT # G-33-W07.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 07/30/90

Project No. G-33-W08 Center No. 10/24-6-R5983-8A0

Project Director MAY S W School/Lab CHEMISTRY

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH

Contract/Grant No. 2 R01 HL28167-08 Contract Entity GTRC

Prime Contract No.

Title NOVEL ANTIHYPERTENSIVES: RATIONAL DESIGN AND EVALUATION

Effective Completion Date 900630 (Performance) 900930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

Comments CONTINUED BY G-33-W09.

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER HL28167	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Dr. Sheldon W. May	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	7/1/89	6/30/90

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Novel Antihypertensives: Rational Design and Evaluation

(SEE INSTRUCTIONS)

1. Plans for the Coming Year

For the coming year our overall objectives remain the same: to answer key questions regarding the biochemical mechanisms responsible for the antihypertensive activity of novel compounds of our design. We plan to continue to pursue the specific aims originally proposed, and no changes are anticipated in our overall experimental strategy.

2. Progress Report for Current Year

This first year of the current project period has been a productive one for our program, with progress having been made towards all of our goals.

Enzymology

Our finding that DBM catalyzes aromatization of 1-(2-aminoethyl)-1,4-cyclohexadiene (CHDEA) a process which proceeds via the normal DBM reductive monooxygenation pathway is significant, since aromatization of cyclohexadienes represents a well established trapping reaction diagnostic of single electron transfer processes. As set out in our publications, our unified mechanism for DBM processing postulates initial single electron transfer from substrate; thus, for example, we visualize that heteroatom cation radicals are initially formed with our N,S or Se substrates. We have determined the kinetic characteristics of CHDEA turnover, and unequivocally proven that the only product from the CHDEA/DBM reaction is 2-phenylethylamine; there is no evidence for any oxygenated products being formed. ^{1}H-NMR, ^{2}H-NMR and GC-MS analysis of the product from paired enzymatic reactions, carried out either in H_2O or in $^{2}H_2O$, demonstrated that no deuterium incorporation into the product, 2-phenylethylamine, occurs during CHDEA/DBM turnover. Dideuterated CHDEA (CHDEA-d_2) was also prepared and examined as a substrate for DBM. The NMR and GC-MS analysis of the product from CHDEA-d_2/DBM reaction revealed no loss of deuterium at the benzylic position of the product occurs. Lack of deuterium loss (or incorporation) effectively rules out any mechanism involving initial hydroxylation at the exocyclic methylene followed by aromatization in DBM/CHDEA reaction, but is consistent with initial abstraction of H from a ring methylene. Since aromatization of cyclohexadienes represents a well established trapping reaction diagnostic of single electron transfer processes, these experiments provide direct support for the ability of the activated copper oxygen species of DBM to carry out a single electron transfer process.

We initiated a pilot evaluation of one class of compounds targeted at the enzyme tyrosine hydroxylase (TH), the monooxygenase which catalyzes hydroxylation of Tyr to dihydroxyphenylalanine (DOPA), thus sitting two steps ahead of DBM in the pathway leading from the essential amino acid, Phe, to the neurotransmitter, NE. In recent work with the enzyme phenylalanine hydroxylase (PAH) we discovered that PAH readily carries out oxygenation of 4-pyridylalanine to the corresponding 4-pyridylalanine-N-Oxide. In the course of this work, we synthesized and fully characterized both various pyridylalanines and their corresponding N-oxides. We therefore reason that TH might similarly carry out conversion of 4-hydroxy-3-pyridylalanine (which exists as its pyridone tautomer) to the corresponding N-oxide (Both PAH and TH are non-heme-iron monooxygenases). This N-oxidation would generate the corresponding pyridone-N-oxide as the product, which is actually a hydroxamic acid N-oxide, an excellent ligand for iron. Thus, the product could readily inhibit TH by ligating to the essential Fe atom in the active site. To date, only weak TH inhibition has been observed with there prototypical compounds, and efforts will continue during the coming year.

Syntheses of DBM-targeted compounds designed based on the Michael acceptor and Pummerer rearrangement strategies outlined in our proposal are underway.

Cell Culture Experiments

We have initiated these studies using the HOPAES derivative, since pilot experiments established that HPLC-EC can be conveniently used to monitor this compound, obviating the need to prepare radiolabeled material. We have now successfully initiated work with primary adrenal cultures. We have now obtained data which clearly establishes time- and concentration-dependent uptake of HOPAES into cells in culture, thus setting the stage for pursuing this important phase of our program.

Bioassay of Antihypertensive Activity.

Our initial attention here has focused on MePAESe, both the racemate and the enantiomers which we separate as described o=in our original proposal. We have now obtained statistically significant data (n=5) showing potent antihypertensive activity for MePAESe in SHR. We have also demonstrated oral activity for MePAESe, an administration route which have not examined heretofore but which is of primary interest from the perspective of clinical potential.

5. Publications.

"Dopamine-beta-Monooxygenase Catalyzed Aromatization of 1-(2-Aminoethyl)-1,4-Cyclohexadiene: Redirection of Specificity and Evidence for a Hydrogen Atom Transfer Mechanism", K. Wimalasena and S.W. May, J. Am. Chem. Soc., 111, 2729-2731 (1989).

"N-Succimidyl Methoxy Phenylacetic Acid Ester, an Amine-Directed Chiral Derivatizing Reagent Suitable for Enzymatic Scale Resolutions", P.A. Husain, J.E. Colbert, S.R. Sirimanne, D.G. VanDerveer, H.H. Herman and S.W. May, Analyt. Biochem, 178, 177-183 (1989).

"Effects of Dopamine b-Monooxygenase Substrate Analogs on Ascorbate Levels and Noreinephrine Synthesis in Adrenal Chromaffin Granule Ghosts", K. Wimalasena, H.H. Herman, and S.W. May, J. Biolog. Chem., 264, 124-130 (1989).

"Interaction of Non-conjugated Olefinic Substrate Analogs with Dopamine-beta-Monooxygenase: Catalysis and Mechanism-Based Inhibition", S. R. Sirimanne and S W. May, Biochemical Journal, submitted (1990).

The Following are Published Abstracts:

Brain Catecholamine Concentrations in the Brindled Mottled Mouse Resemble Those Seen After Inhibition of DBH", Abst. Soc. Neuroscience 15, 98 (1989)

"Hemodynamic Effects of Phenyl-2-Aminoethyl Selenide in WKY and SHR rats", FASEB J. 48, 4669 (1989).

"Effects of Phenyl-2-Aminoethyl Selenide on in-vitro Reactivity to Phenylephrine and BACL2 in Rat Aorta", FASEB J. 48, 4670 (1989).